Tissue tolerance of the canine liver to intraoperative radiotherapy

Cromheecke, Michel

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2006

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 28-12-2018
Chapter 1 describes the aim of the study

In about half of the patients who undergo (successful) surgical treatment for colorectal cancer, metastases to the liver are encountered during or after the operation. Application of external radiotherapy or chemotherapy only has a palliative effect, mainly due to the restrictions of side-effects. Newer therapies, such as cryosurgery and brachytherapy, are often applied to inoperable metastases and they appear to have a favourable effect on survival. At present, various treatment options are receiving consideration, such as radiofrequency ablation, laser-induced thermotherapy, gene therapy and monoclonal antibodies with or without radioactively labelling.

The only treatment for metastases to the liver that has any chance of curing the patient is surgical resection. About 30% of the patients with metastases are eligible for surgical treatment. Five-year survival in these patients is 30-40%. In many of the patients who are candidates for surgical resection of the metastases, it is difficult to achieve completely tumour-free excision margins. These patients have the highest risk of tumour recurrence in the liver.

With the aid of radiotherapy, it is possible to fight the tumour tissue that is left in situ at the surgical margins. External radiotherapy is laborious owing to the high risk of radiation damage to the liver and other sensitive tissues in the region of the liver, such as the intestines. Intraoperative radiotherapy (IORT) comprises the irradiation of the surgical margins with a single high dose of radiation directly following tumour excision. The great advantage of IORT is that the radiation dose can be given at precisely the desired location, while shielding any adjacent sensitive structures.

Presently, it is not clear how liver tissue reacts to a single high dose of radiation. It is obviously not the intention to seriously damage the remaining liver tissue or cause healing deficits or abscess formation that would threaten organ function.

This thesis describes an animal study on the damage that occurs after a single high dose of radiation to normal liver tissue and surgically manipulated liver tissue. The animal model used appeared to be suitable for translation to the human situation.

Chapter 2 describes the basic principles of radiobiology

The effect of exposure to radiation depends on the type of radiation (in this study: electrons) and the energy. During the application of radiotherapy, it is necessary to find a balance between fighting the tumour and causing as little damage as possible to the healthy tissues. Each tissue type reacts differently to radiotherapy. In the case of healthy tissues, the response depends strongly on the rate of cell division. The faster a cell divides, the greater its radiosensitivity.

The type of tissue also plays an important role and this chiefly concerns tissue function (read: organ). In addition, attention should be paid to the exposure volume: if even a very small part of the spinal cord is irradiated, total paralysis can occur. In contrast, parenchymal organs such as the liver usually have sufficient remaining capacity. However, radiotherapy after surgical resection might reduce the remaining capacity drastically.

Characteristic differences in radiosensitivity exist between normal tissue and tumour tissue. Radiobiology makes use of these differences to kill a maximum number of tumour cells while causing the least possible damage to the healthy tissues. By dividing the total radiation dose into small fractions (fractionation) and by applying the fractions over a number of weeks, the highest possible dose can be given with the smallest possible damage to healthy tissues. Owing to the fact that the IORT treatment field does not include healthy tissues, a high dose can be given in one session.

The liver is composed of connective tissue, blood vessels, bile ducts and specialised cells (hepatocytes) that are responsible for e.g. removing waste products from the blood and excreting them from the body in the form of bile via the intestines. All these structures have their own individual radiosensitivity. Hepatocytes can be killed directly by irradiation, or they can die during cell division, or die indirectly at a later date as a result of damage to the connective tissue and blood vessels. Particularly regarding the late effects of radiation damage, it is unclear what the influence is of the single high dose used in this study.
In Chapter 3 the development and application of IORT are described.

IORT is the application of a single high dose of (electron) radiation to an operatively exposed tumour or the surgical margins after tumour resection during an elective surgical procedure. Although the biological effectiveness (RBE) of a single dose is greater than that of fractionated therapy, the advantages of IORT are not so much based on radiobiological principles (see Chapter 2) as on physical principles: the irradiation can be applied very accurately and healthy tissues can be excluded from the treatment field.

IORT can also play a role in the treatment of tumour recurrence after conventional radiotherapy, because IORT spares earlier irradiated normal tissues. In 1907, 12 years after the discovery of X-rays, the first description of IORT was published in connection with an inoperable stomach tumour. A historical overview is given of the development of radiotherapy from the first treatment for skin diseases with X-rays, to the current wide-ranging applications, such as external radiotherapy with 3-dimensional planning, brachytherapy, radioactive labelled antibodies and IORT. It speaks for itself that the results of animal experiments and clinical studies in combination with rapid technological progress have increased the capacity of radiotherapy over the past 100 years.

In the period from 1964 to 1976, the first clinical studies were performed in Japan on patients treated with IORT. Soon afterwards, studies were performed in the US, Spain and Germany. Since 1986, IORT has been used in clinical studies in the Netherlands (Groningen, Eindhoven) mainly for colorectal cancer. In many cases, a combination of surgery, external radiotherapy and IORT was used.

These clinical studies were preceded by extensive animal experiments on the sensitivity of various tissues and organs to a single high dose of radiotherapy. The dog proved to be a reliable and comparable animal model to translate the results to the human situation.

At present, IORT is being applied to e.g. gastrointestinal tumours, breast cancer, sarcomas, pancreatic and biliary tumours and head and neck tumours. Although various studies have indicated a positive effect on survival in gastrointestinal cancer patients, similar effects have not yet been proven at other locations. Well-known problems are the difficulty of setting up prospective randomised trials and the need for multicentre studies: only a few centres are able to apply IORT, owing to the need for large infrastructural investments.

Chapter 4 describes the current treatment for colorectal liver metastases.

The only treatment for liver metastases that has a chance of curing the patient is surgical resection. Increasing experience and improved diagnostics, indications, surgical techniques and perioperative care have resulted in a 5-year survival rate of 21 to 48%.

Nevertheless only 25% of patients with liver metastases are suitable candidates for surgical treatment. Patient selection is extremely important in the decision of whether or not to perform surgical excision of liver metastases.

Prognostic factors in the surgical treatment for liver metastases chiefly concern: number and size of the metastases, metastases outside the liver and the width of the tumour-free surgical margins.

Treatment for liver metastases with chemotherapy, whether or not in combination with other treatments, still only has a palliative effect. Studies on different combinations of antitumour therapies applied according to different protocols showed no more than slight improvement in survival. One of the limiting factors was the severe side-effects of chemotherapy for the patient. Experimental techniques for the administration of antitumour medication, such as isolated liver perfusion or labelled antibodies, may help to solve this problem.

Tailored direct treatment for metastases, such as freezing (cryotherapy) or destroying them by means of...
heat (radiofrequency ablation and laser-induced thermotheraphy), seems to have a favourable effect on inoperable metas-
tases, although at present there are insufficient data.

Despite improved techniques, conventional radiotherapy in the form of external irradiation only has palliative value owing to the high risk of damage to normal tissues. Other treatment strategies that make use of radiation are currently under study. These include remote afterloading interstitial (Ir 192) radiotherapy, radioactive implants, the use of radioactive labelled anti-
body complexes and selective embolisation by means of radioactive colloids.

Chapter 5 gives an overview of current knowledge on radiation damage to the liver.

Animal studies and clinical studies have shown that the radiosensitivity of the liver varies depending on the physical pa-
rameters of the radiation, such as type of irradiation, dose, fractionation and treatment volume. In addition, the physical
status of the patient and his/her liver are of importance. The use of toxic medication and surgery to the liver lead to greater
radiosensitivity.

In patients, radiation damage is chiefly expressed in a specific type of liver inflammation that can recover or ultimately cause
organ failure. Generally, it can be concluded that fractionated radiotherapy (maximal 3 Gy fractions) with a total dose of 30
Gy can be applied to the whole liver without any clear functional damage.

Chapter 6 describes the experimental animal model used in this study

Previous tissue tolerance studies have shown that the dog as experimental animal is a reliable model, because the
technical procedure can be translated accurately to the human situation owing to the size of the animal. Structural and histo-
lological similarities between the canine liver and the human liver mean that the outcomes of this study can be used for clinical application.

A group of 25 beagles were divided into 5 subgroups based on the radiation dose received. Each animal un-
derwent laparotomy and partial liver lobe resection (about 50% of the total volume). Haemostasis of the surgical
margins was achieved using compression stitches. In the same session, the surgical margins and the non-surgically manipulat-
ed liver lobe were irradiated using a Linear Accelerator (electron energy 10 MeV) with 10 Gy, 20 Gy, 25 Gy or 30
Gy. In each subgroup, one animal served as a control by undergoing the total surgical and irradiation procedure with a dose of 0 Gy. The radiation was applied accurately to the desired location on the liver (the left lobe) using a special tube fitted to the linear accelerator. Lead shielding was used to exclude adjacent structures from the treatment volume, e.g. intestines and pancreas.

Medical files were kept on the experimental animals and blood tests were performed regularly.

After 3 months, 1 year, 2 years, 3 years and 5 years, ani-
Chapter 7 presents the results at 1-year follow-up

The combined surgical and irradiation procedures were conducted satisfactorily. During the follow-up period of 1 year, the experimental animals survived the treatment well. Only one animal developed super-
ficial wound infection, which responded to antibiotic treatment. Blood tests showed that in the majority of animals, the haemoglobin value decreased postoperatively, but the values remained within the normal range and normalised to the preoperative lev-
els within 3 weeks. During the first 6 weeks after the operation, alkaline phosphatase increased in some cases to 10 times the normal value. This parameter for hepatocyte damage was not dose-dependent: the control group also showed increased levels. Within 6 months, the values had normalised. It is probable that the increases were more the result of partial liver resection than the radiotherapy. No further liver func-
tion disturbances were observed.

At 3 months and at 1 year, one animal in each dosage group was killed for evaluation. It appeared that the resection margin of the liver had adhered to the peritoneum and there was considerable fibrosis (scar tissue). After 3 months, the radiotherapy site showed a grey area, which under the microscope proved to be thickening of the liver capsule. After 1 year follow-
up, this was characterised by a local defect in the liver tissue in combination with severe fibrosis. These findings were even observed after the lowest dose.
of 10 Gy. No abnormalities were found in the other abdominal organs. Histopathological changes were present, such as interstitial fibrosis, hepatocyte atrophy, biliary duct proliferation and an inflammatory reaction of the parenchyma. These abnormalities increased with time and with the size of the radiation dose; after 1 year, only the parenchymal inflammatory reaction had disappeared.

Electron microscopic examination after 1 year showed interstitial fibrosis of the parenchyma just beneath the capsule and balloon-shaped abnormal hepatocytes in the irradiated area. No relation could be established with the radiation dose applied.

It could be concluded that in this animal model, intraoperative radiotherapy of the liver was easily feasible without any perioperative complications. Very little histological damage was visible after 3 months, except for one parenchymal inflammatory reaction, which suggested high resistance in the short-term. However, after 1 year, severe parenchymal abnormalities were found within the irradiated area.

Chapter 8 presents the results of scintigraphy that aimed to detect and monitor possible liver damage in a noninvasive manner.

Radioactive technetium-labelled (isotope: 99mTc) sulphur colloid was injected intravenously and absorbed selectively by the Kupffer cells. Any poorly functioning Kupffer cells will show poor technetium absorption. Graphic images of the liver were obtained using a gamma camera. Areas with decreased absorption were visible as less intense areas on the scans and a scoring system was applied. Liver scans were performed preoperatively and at 1, 3 and 6 weeks postoperatively and repeated at regular intervals during follow-up.

The liver scans were judged for decreased or absent (shrivelled) absorption capacity, changes in size (enlargement) of the irradiated area.

An independent nuclear medicine specialist read the scans. The abnormalities on the scans were poorly correlated with the abnormalities found at autopsy. Absent or decreased absorption in the long-term were the only features that appeared to be consistently dose-dependent. Possible explanations were that the target area was relatively small compared to the total volume of the liver and the abnormalities were located in the periphery. In addition, movement artefacts due to respiration may have hindered reliable interpretation.

Chapter 9 presents the long-term results of the study.

All the experimental animals remained in good physical condition to the end of follow-up. At 2 years, 3 years and 5 years after the IORT procedure, the remaining subgroups were killed for histopathological evaluation. It was striking that after 2 years, the macroscopic defect was smaller and at 5 years, it had almost disappeared. Microscopic examination showed that the thickening of the capsule in the short-term had decreased, but that the subcapsular fibrosis had increased. Biliary duct proliferation and hepatocyte atrophy had decreased. A characteristic finding was arteriolar changes, such as endothelial proliferation and (after 5 years) focal hyalinosis with stenosis of the lumen. Periportal fibrosis that led to bridging was maximal after 2 and 3 years, but had decreased after 5 years. A clear border could be distinguished between fibrotic parenchyma and normal parenchyma.

After partial liver resection in this animal model, it proved to be technically feasible to apply a single high dose of electron radiation to the resection margins and to an area of the normal liver. During a follow-up of 5 years, there were no cases of treatment-dependent morbidity or mortality in our experimental animals. Short-term and long-term radiation damage was considerable. It was initially expressed in the form of hepatocyte atrophy and biliary duct proliferation and later expressed in parenchymal fibrosis and after 5 years, in portal vein abnormalities.

In view of the results of this study and previous animal experiments using IORT, this animal model seems to be suitable for translation to the clinical situation.
Concluding remarks

The incidence of colorectal carcinoma is still increasing. About 50 - 70% of the patients develop local recurrence or metastases to the liver and/or lungs. During many years of clinical application to treat local recurrence, the combination of surgical resection and IORT has proved to have favourable results in randomised studies. It has not yet been clearly established whether IORT also has favourable effects on survival on other malignancies, such as pancreas carcinoma and gastric carcinoma although local tumour control seems to be improved. One of the reasons for this is that adequate studies are lacking. Possibilities for the application of IORT seem to regard the treatment of breast cancer. Future treatment of breast cancer could consist of lumpectomy and a sentinel node procedure followed by intraoperative radiotherapy of the biopsy site without adjuvant external beam irradiation of the whole breast. Local treatment for liver metastases does not offer a solution for a disease that disseminates haematogenically. Only 30% of the patients with colorectal metastases to the liver are suitable candidates for resection. High recurrence rates in and outside the liver mean that adjuvant treatment is necessary. Systemic and regional chemotherapy have palliative value alone or lead to slight benefit in combination with surgery. Precise targeting of the radiation beam in new external radiotherapy techniques has successfully decreased the risk of damage to healthy tissues. New local and systemic treatment approaches became recently available. A new systemic treatment approach is the so-called "targeted therapy"; a combination treatment of an antibody against epidermal growth factor-receptor (EGF-R, cetuximab) and a vascular endothelial growth factor (VEGF, bevacizumab) both in combination with chemotherapy. Pharmacogenetics might also play a role in the near future in the selection of patients who are candidates for a specific chemotherapy treatment. Cryosurgery of liver metastases has not yielded the desired results. Radiofrequency ablation seems promising, but is so far chiefly useful as local (palliative) treatment for inoperable metastases. Surgical resection of liver metastases, with or without RFA, in case of marginal resection, combined with precise target radiation treatment, as well as the use of new regional or systemic chemotherapy approaches might alter the disease outcome. The final goal of the combined treatment approaches in the fight against metastatic colorectal cancer to the liver is reducing local and distant failure and therefore improving disease free and overall survival.